Application Note

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More Sensitive Quantification of PFAS by LC/MS with the Agilent 1260 Infinity II Hybrid Multisampler

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Abstract

This application note demonstrates the use of the Agilent 1260 Infinity II Hybrid Multisampler for the analysis of per- and polyfluorinated alkyl substances (PFAS). The Hybrid Multisampler in feed injection mode was used with optimized Feed Speed for the sample injection to trap and enrich the compounds on the column. This avoided peak broadening and breakthrough of the early-eluting polar PFAS compounds. The less polar PFAS compounds can be enriched for more sensitive quantification.

Introduction

The analysis of PFAS often requires sample preparation techniques, such as solid phase extraction, whenever low detection limits are required. The final samples ready for LC/MS/MS analysis are therefore usually dissolved in 80 to 100% organic solvents.[1] Additionally, recommended concentration limits have gotten even lower in recent years.[2] Injecting high sample volumes could improve sensitivity and therefore allow lower detection limits, but this is limited by undesirable solvent effects caused by the high elution strength of the sample solvent in common reversed-phase liquid chromatography. Even though these limitations can be mitigated to some extent by adding a significant amount of a more polar stationary phase (such as a diol cartridge) upstream of the separation column, or performing a sandwich injection, peak shapes are still affected at high injection volumes.[3]

This application note presents the use of feed injection as an alternative to the common flow-through injection. This allows for much higher injection volumes without a negative impact on the peak shape, unlike flow-through injections, even when the sample is dissolved in 100% organic solvents. This improvement is achieved by infusing the sample into the mobile phase stream with a special valve, resulting in an online dilution. This functionality is implemented in the Agilent 1260 Infinity II Hybrid Multisampler.[4] The use of a novel C18 reversed-phase column designed to be compatible with a 100% aqueous mobile phase helps to maximize the improvement.

Experimental

An LC/MS system was used, converted for low PFAS background with the Agilent PFC-Free HPLC Conversion Kit (5004-0006).[5] The system consisted of:

- Agilent 1290 Infinity II High Speed Pump (G7120A)
- Agilent 1260 Infinity II Hybrid Multisampler (G7167C)
- Agilent 1290 Infinity II Multicolumn Thermostat (G7116B)
- Agilent Ultivo Triple Quadrupole LC/MS (G6465B)

Column

Agilent InfinityLab Poroshell 120 Aq-C18, 2.1 × 100 mm, 2.7 µm (part number 695775-742)

Guard column

Agilent InfinityLab Poroshell 120 Aq-C18, 2.1 mm, 2.7 µm (part number 821725-955)

Delay column

Agilent InfinityLab PFC delay column, 4.6 × 30 mm (part number 5062-8100)

Software

- Agilent MassHunter Acquisition Software, version 1.2
- Agilent MassHunter Quantitative Software, version 10
- Agilent MassHunter Qualitative Software, version 10

Method

LC/MS/MS Method	
Mobile Phase	A) Water with 5 mM ammonium acetate B) Methanol
Flow Rate	0.4 mL/min
Gradient	Time (min) %B 0 to 1 0 1 to 2 0 to 50 2 to 6 50 to 70 6 to 7.5 70 to 80 7.5 to 12.5 80 to 100 12.5 to 14.9 100 14.9 to 15.0 100 to 0 – Run time: 17.5 min – Post time: Off
Flow-Through Injection	– Draw speed: 100 µL/min – Injection volumes: 5, 7.5, 10, 15, 20, 30, and 40 µL
Feed Injection	– Feed speed: 10% of flow (adaptive) – Injection volumes: 5, 7.5, 10, 15, 20, 30, 40 µL – Automatic overfeed volume – Flushout solvent: mobile phase A – Wash solvent: mobile phase B – Inner/outer wash: 150 µL/5 s – Reconditioning with mobile phase A
Column Temperature	55 °C
MS Settings	
The Agilent PFAS MRM database for triple quadrupole LC/MS (G1736AA) was used for all LC/MS settings, without modifications, except retention time settings.	
MS Source Parameters	
Drying Gas	230 °C, Flow: 5 L/min
Nebulizer Pressure	15 psi
Sheath Gas	350 °C, Flow: 11 L/min
Capillary Voltage	–2,500 V
Nozzle Voltage	0 V
Polarity	Negative

Standards

47 PFAS analytes (PFBA, PFPeA, PFHxA, PFHpA, PFOA, PFNA, PFDA, PFUnDA, PFDoDA, PFTrDA, PFTeDA, PFHxDA, PFODA, PFBS, PFPeS, PFHxS, PFHpS, PFOS, PFNS, PFDS, PFDoS, FBSA, FHxSA, FOSA, HFPO-DA, MeFOSAA, EtFOSAA, 4:2FTS, 6:2FTS, 8:2FTS, ADONA, 9Cl-PF_3ONS, 11Cl-PF_3OUnS, PFMPA, PFMBA, NFDHA, PFEESA, MeFOSA, EtFOSA, MeFOSE, EtFOSE, 3:3FTCA, 5:3FTCA, 7:3FTCA, 8:2FTUCA, PFecHxS, and 8:2diPAP) dissolved in 99.6% MeOH:0.4% water or 90% ACN:9.6% MeOH:0.4% water, respectively, at concentrations ranging from 0.7 to 10 pg/µL.

Solvents and chemicals

LC/MS grade solvents:

- Agilent InfinityLab water for LC/MS (5191-5121)*
- Agilent InfinityLab methanol for LC/MS (5191-5111)*
- Agilent InfinityLab acetonitrile for LC/MS (5191-5101)*

Ammonium acetate was purchased from VWR, Germany. PFAS standards were purchased from Wellington Laboratories, Canada.

Results and discussion

Flow-through injection

The 1260 Infinity II Hybrid Multisampler can be operated in two modes, either the classic flow-through injection hardware setup, or the feed injection mode, where the sample is infused into the mobile phase stream during injection (Figure 1).

Running the flow-through injection mode with 5 µL injection volume of a PFAS mixture dissolved in 99.6% MeOH resulted in chromatograms with very good peak shapes, except in the case of perfluorobutanoic acid (PFBA, 3.47 minutes), where a significant shoulder was observed, caused by the high elution strength of the sample solvent. No breakthrough occurred at 5 µL injection volume (Figure 2). Increasing the injection volume above 5 µL caused only a minor increase in peak heights. At the same time, higher injection volumes had a significant negative impact on peak shapes (Figure 3).

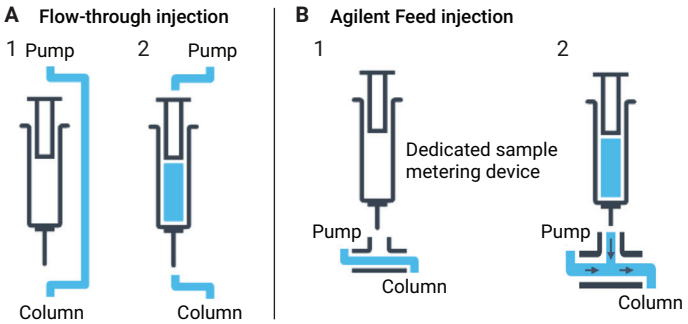

Figure 1. Classic flow-through injection (A) versus feed injection (B).

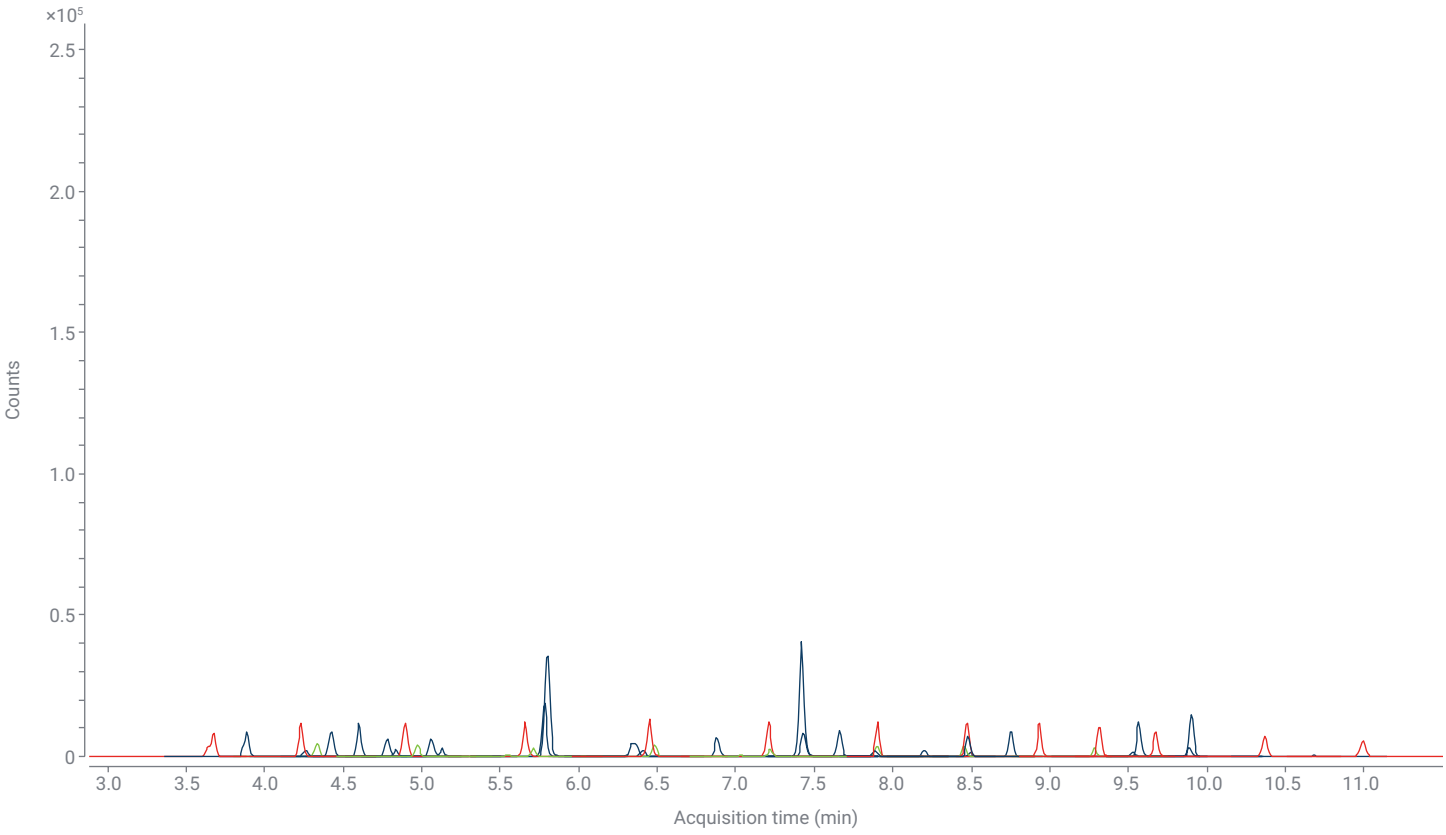

Figure 2. Mixture of 47 PFAS (red: perfluoro carbonic acids (PFCAs), green: perfluoro sulfonic acids (PFSAs), blue: others; quantifier transitions; 5 µL injection volume).

* Only available in select countries.

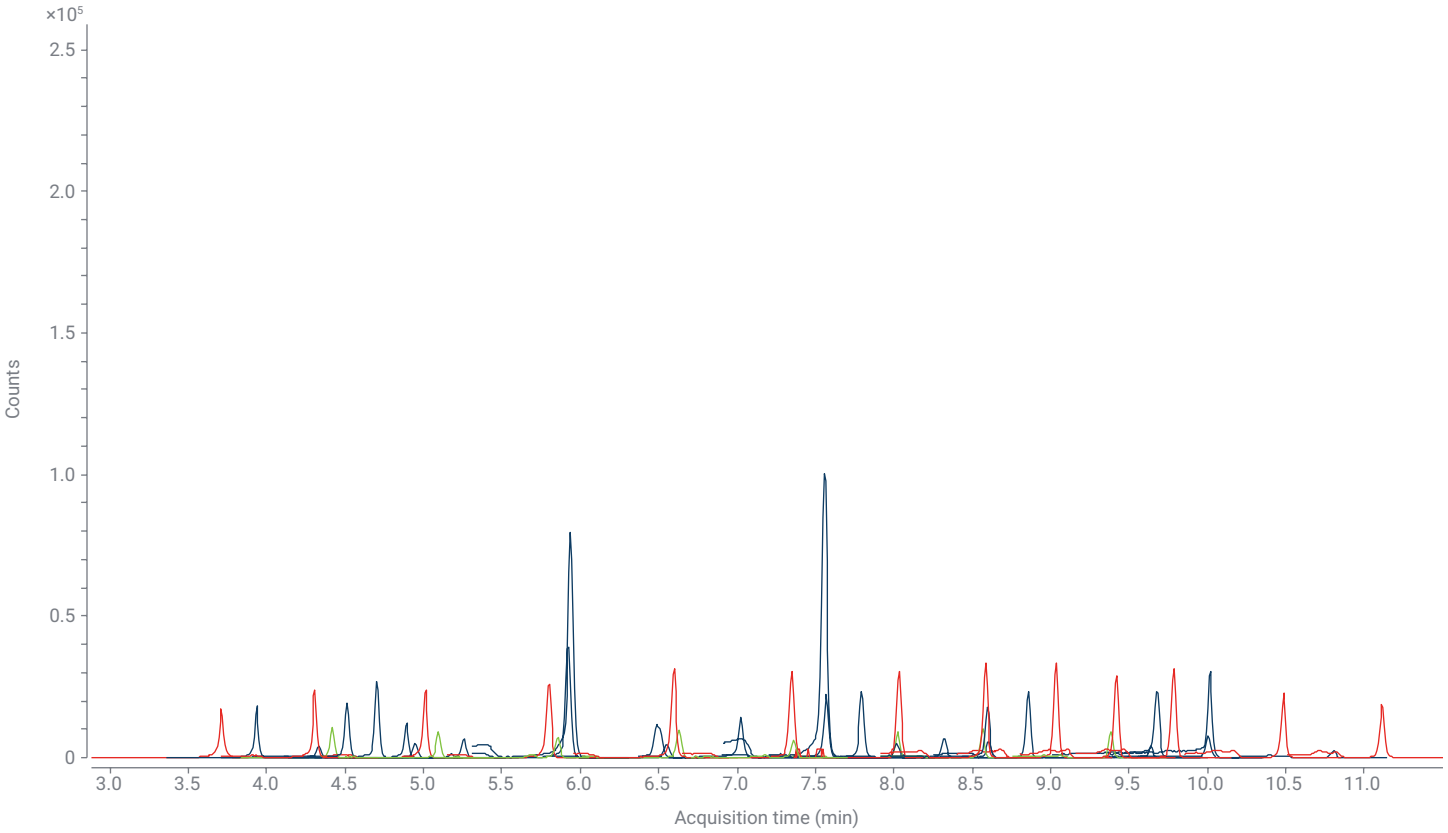

Figure 3. Mixture of 47 PFAS (red: PFCAs, green: PFSAs, blue: others; quantifier transitions; 40 µL injection volume).

Shoulders appeared, peaks were split, and significant fronting was observed. These peak distortions caused issues with automatic integration, which made manual integration necessary and can interfere with the detection and quantification of branched PFAS isomers.

Feed injection

For feed injection, a slow feed speed of 10% was chosen, resulting in a 1:10 dilution of the sample with the starting composition of the method during injection. The InfinityLab Poroshell 120 Aq-C18 column was used, and it was allowed to run at 100% aqueous. This resulted in a 90% aqueous composition during injection. Most of the compounds were therefore trapped and focused when entering the column, and only eluted with the increasing organic ratio.

Even at 40 µL injection volume, very good peak shapes were observed, except for a few compounds eluting very early, showing some peak-broadening. However, no breakthrough was observed for any of the analytes. Compared to the flow-through injection, sensitivity was significantly increased, allowing for lower detection limits (Figure 4).

Retention times are lower for feed injection, since the delay volume of the sample volume is not in the gradient flow path as it is for flow-through injection.

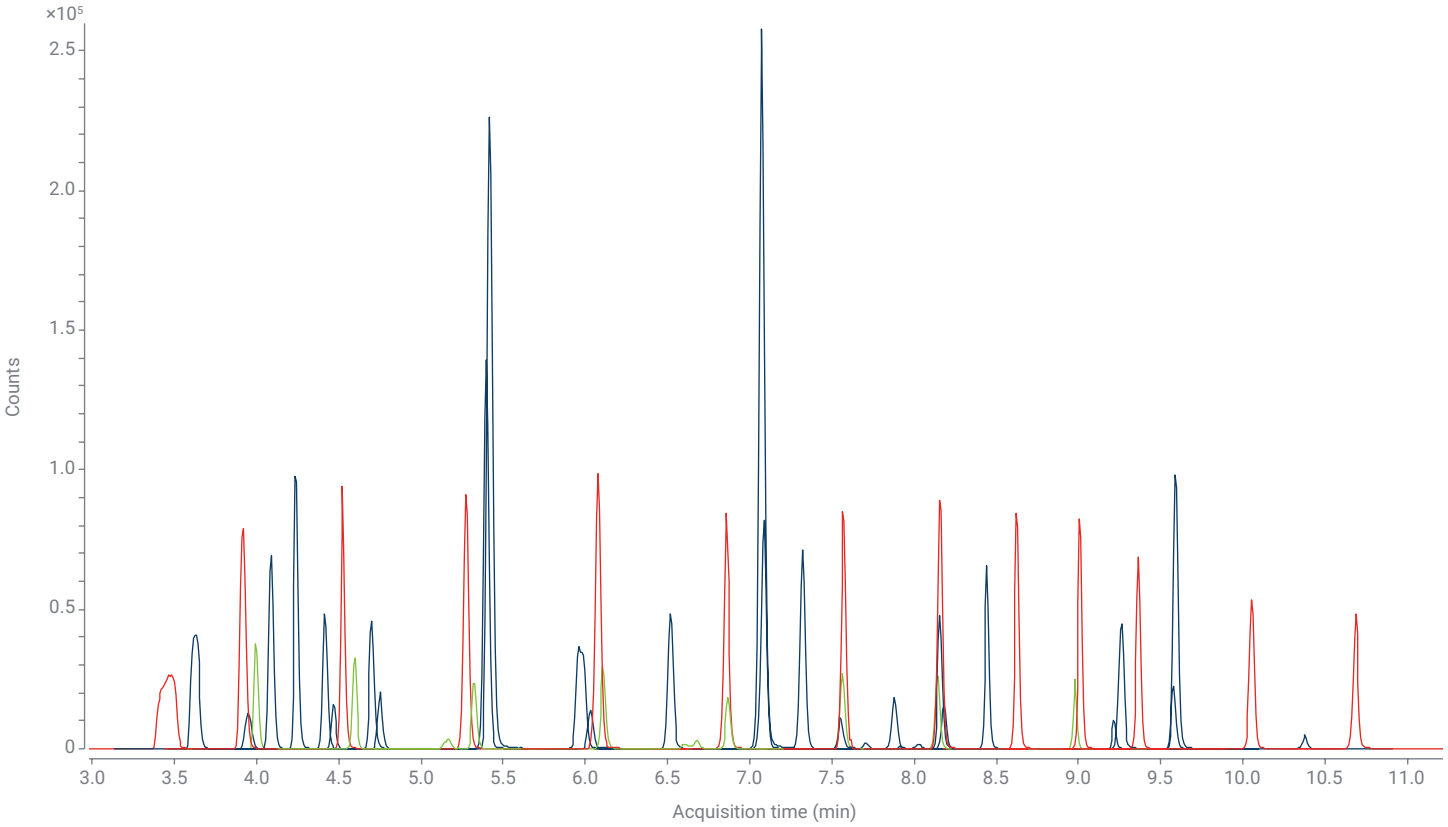

Figure 4. Mixture of 47 PFAS (red: PFCAs, green: PFSAs, blue: others; quantifier transitions; 40 µL injection volume by means of feed injection).

Detailed comparisons for select analytes

The following chromatograms and graphs show detailed comparisons of select PFAS analytes. The chosen analytes include the first eluting compound (PFBA) of the mixture used (Figure 5), two analytes with branched isomers present (PFHxS and EtFOSAA, Figures 6 and 7), and the last eluting compound, PFODA (Figure 8). When increasing injection volumes in flow-through mode, the strong eluting sample solvent causes increasingly severe issues in chromatography. Peaks start to form shoulders early, before they split, at higher injection volumes. Parts of the split peaks can also overlap with the elution of branched isomers, negatively impacting the ability to detect and quantify them (flow-through mode chromatograms in Figures 6 and 7). In case of feed injection, this effect is not observed. Therefore, branched isomers can be detected and quantified without issue. In the case of flow-through injection, breakthrough increases with injection volume. This is most dominant in the case of the most polar, early eluting analytes, such as PFBA (Figure 5), but also present in case of the least polar, late-eluting analytes such as PFOcDA (Figure 8). This impacts the results by only gaining a very limited increase of peak intensity and peak area with increasing injection volumes in flow-through injection mode. In feed injection mode, there is a perfectly linear correlation between injection volume and peak height in almost all cases (Figures 6 to 8), except the earliest-eluting analytes. However, no breakthrough is observed and therefore, feed injection provides a linear correlation between peak areas and injection volumes for all analytes investigated here, even up to the 40 µL injection volume (Table 1). In general, the use of feed injection mode improves peak shape and peak height, while in classical flow-through mode, peaks become broader, are split, and break through.

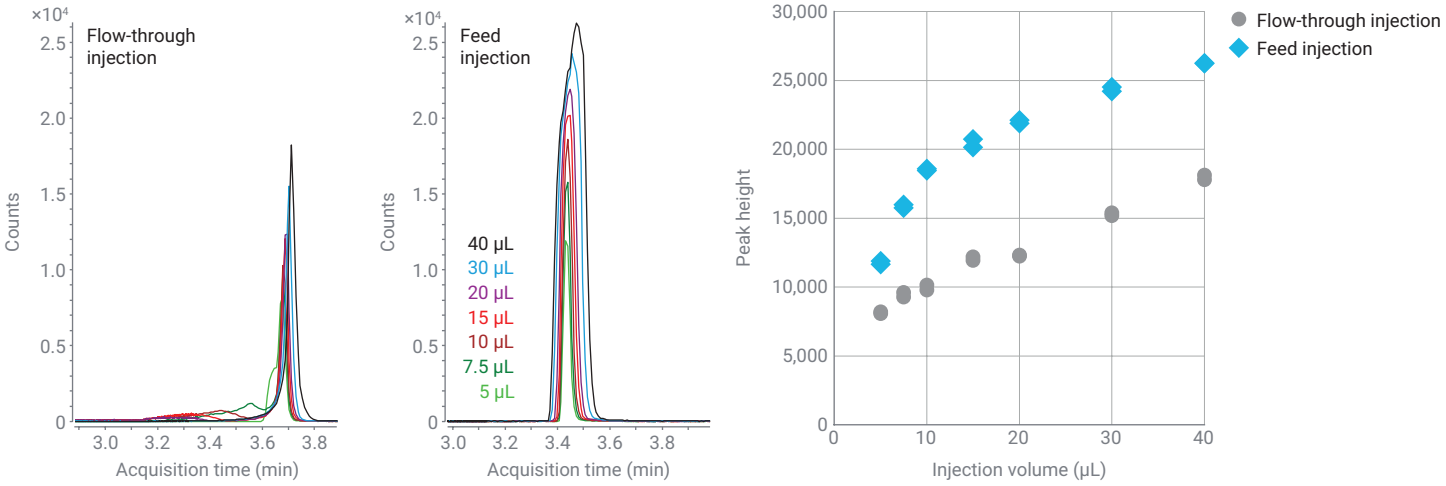

Figure 5. PFBA (comparison of flow-through injection (grey) with feed injection (blue)).

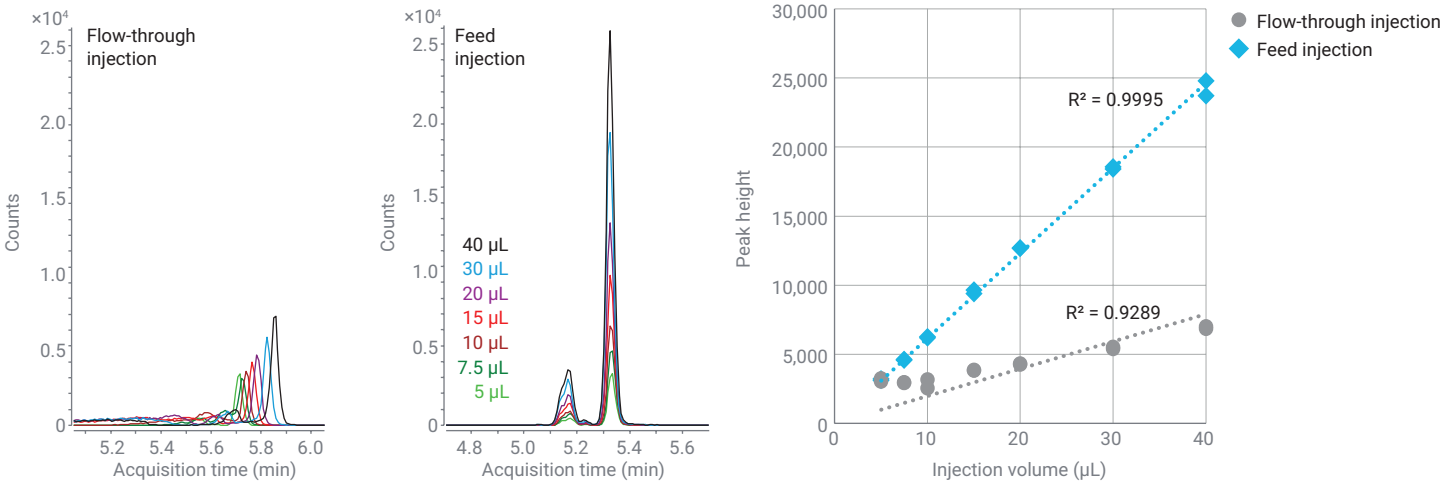

Figure 6. PFHxS (perfluorohexanesulfonic acid, comparison of flow-through injection (grey) with feed injection (blue)).

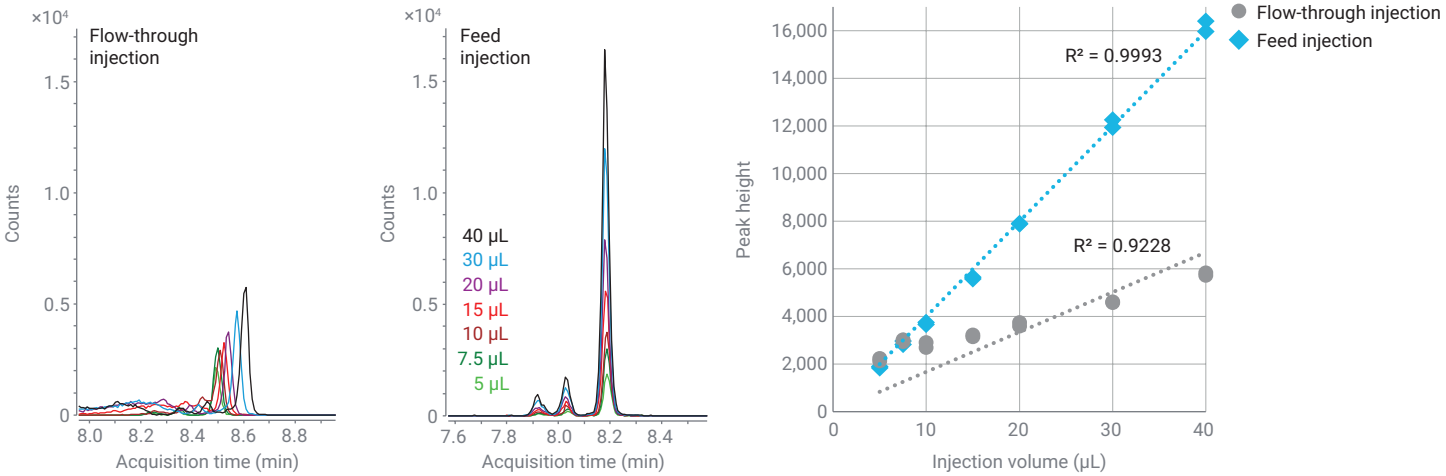

Figure 7. EtFOSAA (N-ethylperfluorooctanesulfonamidoacetic acid, comparison of flow-through injection (grey) with feed injection (blue)).

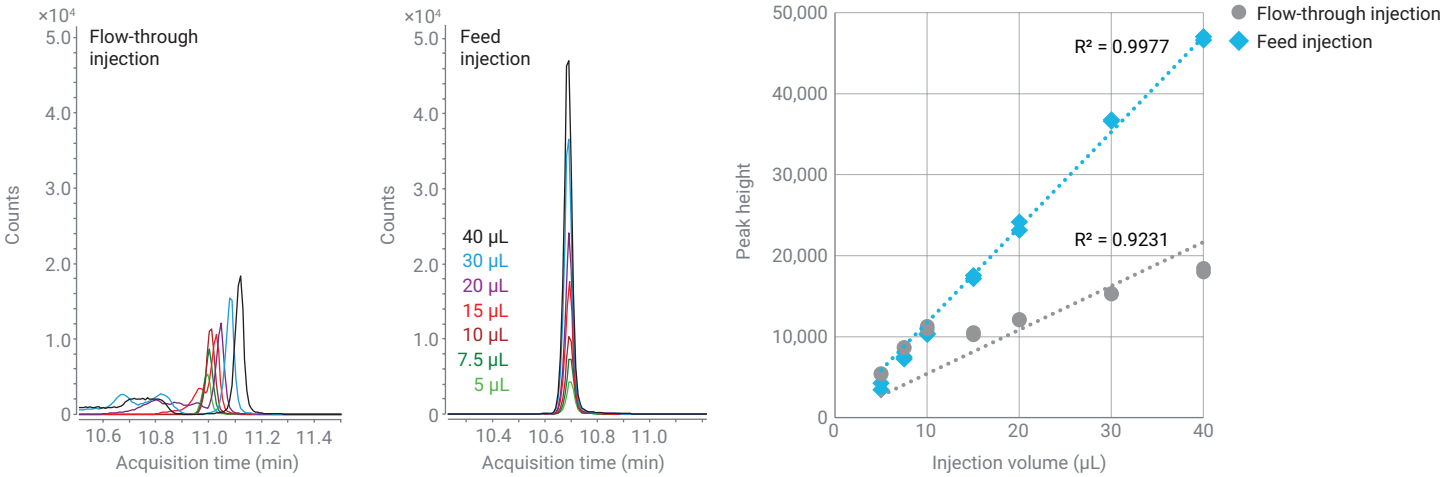

Figure 8. PFODA (perfluorooctadecanoic acid, comparison of flow-through injection (grey) with feed injection (blue)).

An explanation for the peak broadening in the earliest eluting compounds could be based on retention factors, determined in an isocratic run with 10% mobile phase B, which equals the composition during the injection. At very high retention factors (> 20), the analytes are trapped when entering the column and only elute when the mobile phase B ratio increases. Therefore, sharp peaks were observed. With decreasing retention factors, this effect decreases and peak broadening increases. The correlation of peak area versus injection volume is perfectly linear in the case of feed injection, and better sensitivity can be achieved compared to flow-through injection. Only at low retention factors (< 5), like for PFBA, feed injection with high injection volumes might not be as beneficial, as in the case of later-eluting analytes.

Typical values for the peak area linearity for feed injection were ≥ 0.99, while for flow-through injection they were ≥ 0.9 (Table 1). The peak area RSDs were typically in the range of 0.24 to 1.31 for 40 µL by feed injection, and 0.37 to 6.70 by means of flow-through injection. Comparing flow-through and feed injection, the peak height increase was typically 2.1 to 3.7 for 40 µL injection volume, and even higher when 40 µL feed injection is compared to the more realistically useable 5 µL injection volume in flow-through injection (3.2 to 10.9).

Table 1. Summary of results obtained from typical PFAS compounds.

Analyte Retention Time (min)	Retention factor k' at 10% MPB isocratic elution	Linearity R^2 Peak Height vs. Injection Volume, (Forced Through Zero)		Linearity R^2 Peak Area vs. Injection Volume (Forced Through Zero)		Peak Area %RSD		Peak Height		Peak Height Increase Factor	
		Feed Injection	Flow-through Injection	Feed Injection	Flow-through Injection	Feed Injection (40 µL, n = 6)	Flow-through Injection (40/5 µL, n = 6)	Feed Injection (40 µL)	Flow-through Injection (40/5 µL)	Feed Injection vs. Flow-through Injection (40 µL)	Feed Injection (40 µL) vs. Flow-through Injection (5 µL)
PFBA (3.47)	4.8	0.866	0.899	0.999	0.909	0.49	0.54/0.26	26.6 k	17.6/8.28 k	1.5	3.2
PFMPA (3.62)	8.9	0.927	0.908	1.000	0.915	0.61	0.28/0.82	40.6 k	17.8/8.32 k	2.3	4.9
PFPeA (3.92)	24	1.000	0.908	1.000	0.916	0.41	0.94/0.53	78.1 k	24.0/11.4 k	3.3	6.8
3:3 FTCA (3.95)	20	0.971	0.913	1.000	0.879	0.43	3.26/1.62	12.6 k	4.11/1.97 k	3.1	6.4
PFBS (3.99)	45	0.996	0.926	1.000	0.924	0.82	0.99/1.32	37.6 k	10.4/4.43 k	3.6	8.5
PFMBA (4.09)	39	0.995	0.918	1.000	0.914	0.59	1.18/0.69	68.2 k	19.3/8.61 k	3.5	7.9
PFEESA (4.24)	91	0.999	0.924	1.000	0.923	0.77	1.12/0.79	100 k	26.6/11.6 k	3.8	8.6
NFDHA (4.41)	N/A	0.999	0.916	1.000	0.928	1.26	0.89/1.02	48.4 k	12.9/5.80 k	3.7	8.3
4:2 FTS (4.46)	N/A	0.993	0.907	0.997	0.921	0.99	129/1.61	16.1 k	5.01/2.40 k	3.2	6.7
PFHxA (4.52)	N/A	0.999	0.913	1.000	0.929	0.54	1.57/0.71	92.8 k	23.9/11.4 k	3.9	8.2
All Others (4.59 to 10.69)	N/A	0.988 to 1.000, MeFOSE: 0.969	0.884 to 0.980, MeFOSE: 0.805	0.984 to 1.000, MeFOSE: 0.975	0.826 to 0.950, MeFOSE: 0.781	0.24 to 1.31 (NFDHA 5.27)	0.37 to 6.70, MeOFSE: 10.6/ 0.36 to 3.26, HFPO-DA: 4.19	4.81 to 259 k	2.29 to 1.00/0.44 to 41.3 k	2.1 to 3.7	3.8 to 10.9

Acetonitrile as sample solvent

When using a high concentration of acetonitrile in the sample solvent, similar results can be obtained. Due to the higher elution strength of acetonitrile compared to methanol in reversed-phase LC applications, more peak broadening of the earliest-eluting PFAS is observed. Still, the use of feed injection is at least as beneficial in this case, especially as the peak distortion from solvent effects is more pronounced.

At 2.5 µL, flow-through injection performs well, with only a slight but unproblematic shoulder in case of PFBA (Figure 9). Increasing the injection volume to 5 µL already causes significant peak distortion and splitting for high polarity analytes and shoulders in the case of medium polarity analytes, due to the high elution strength of acetonitrile (Figure 10).

Using feed injection, no negative impact on peak shape was observed for injection volumes identical to flow-through injections (Figures 10 and 11). In feed injection mode, even injection volumes up to 40 µL are possible without any breakthrough (Figure 12). The peak broadening of the most polar analytes is slightly more pronounced compared to samples dissolved in methanol.

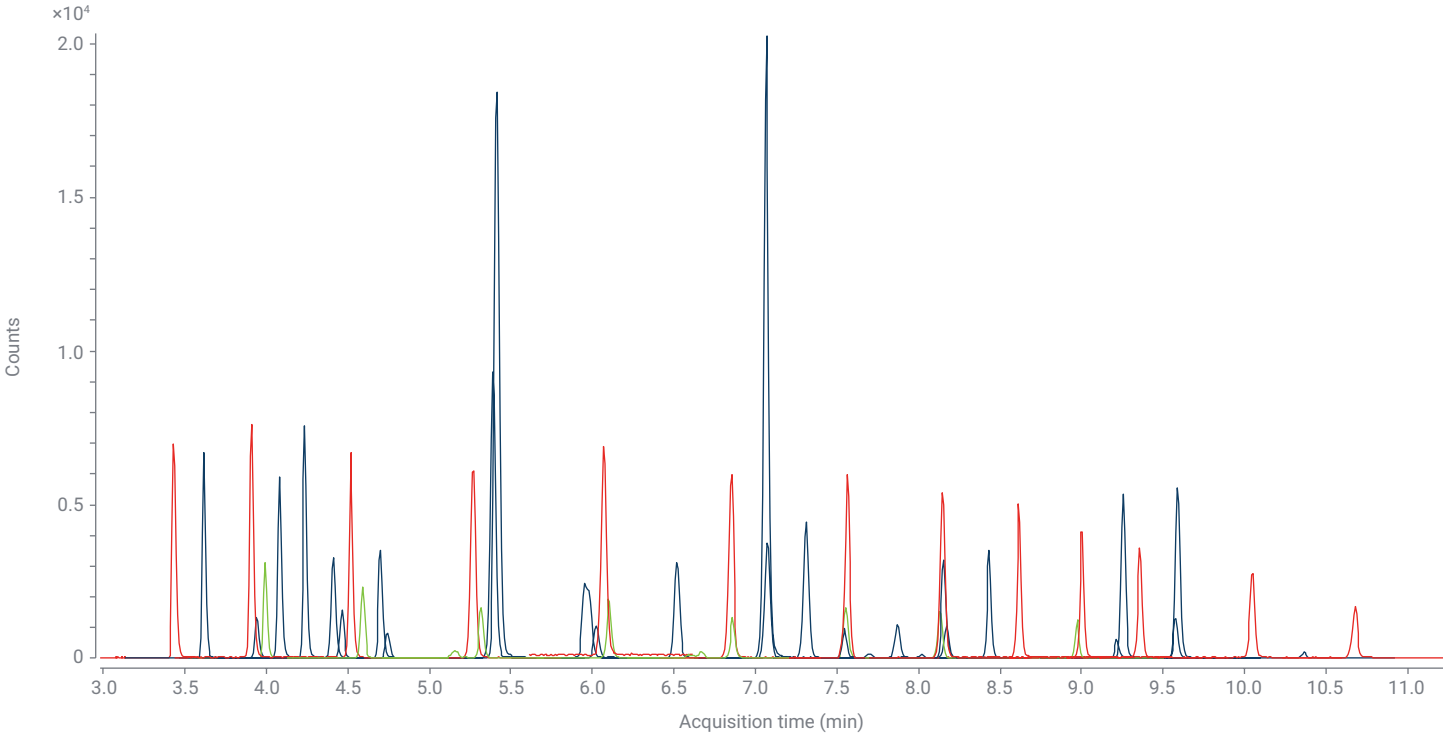

Figure 9. Mixture of 47 PFAS in 90% ACN, 9.6% MeOH, 0.4% water (red: PFCAs, green: PFSAs, blue: others; quantifier transitions; 2.5 µL injection volume, flow-through injection).

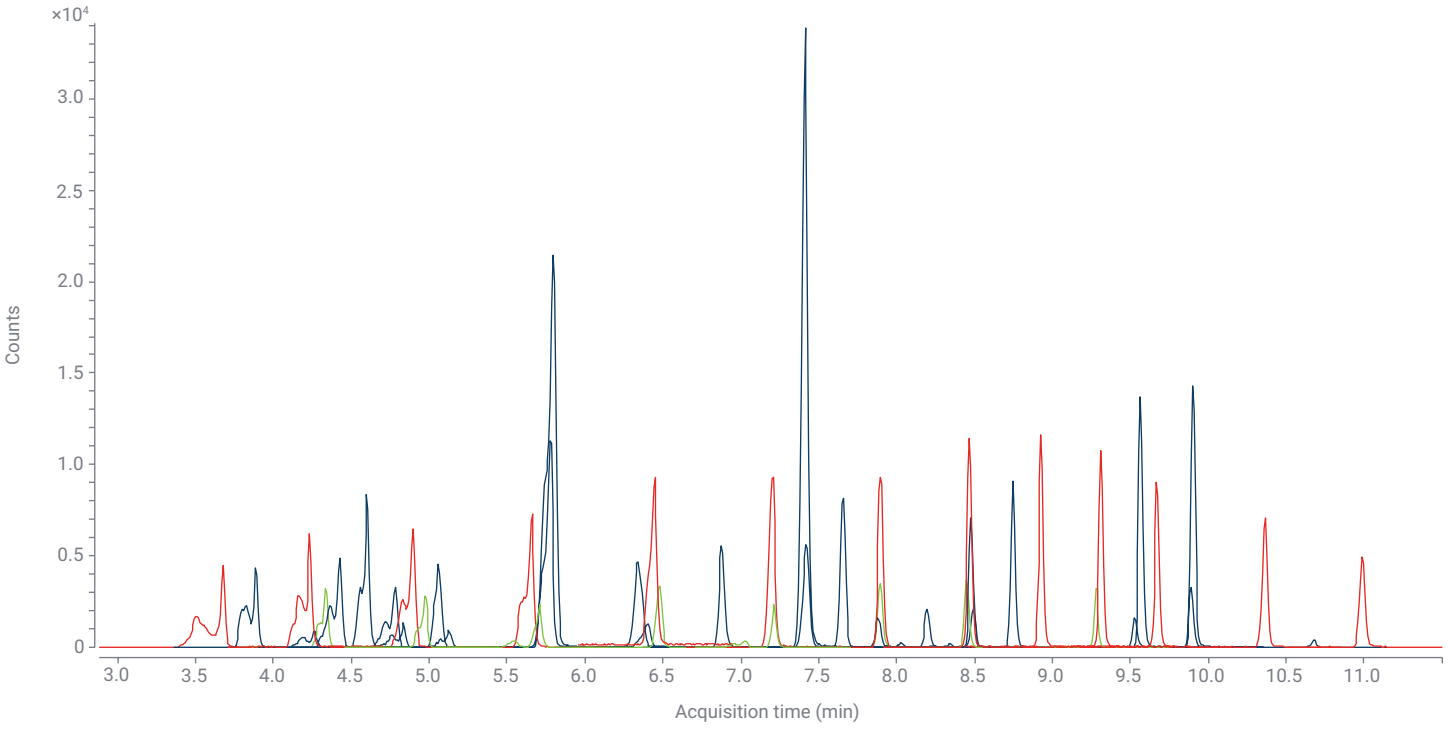

Figure 10. Mixture of 47 PFAS in 90% ACN, 9.6% MeOH, 0.4% water (red: PFCAs, green: PFSAs, blue: others; quantifier transitions; 5 µL injection volume; flow-through injection).

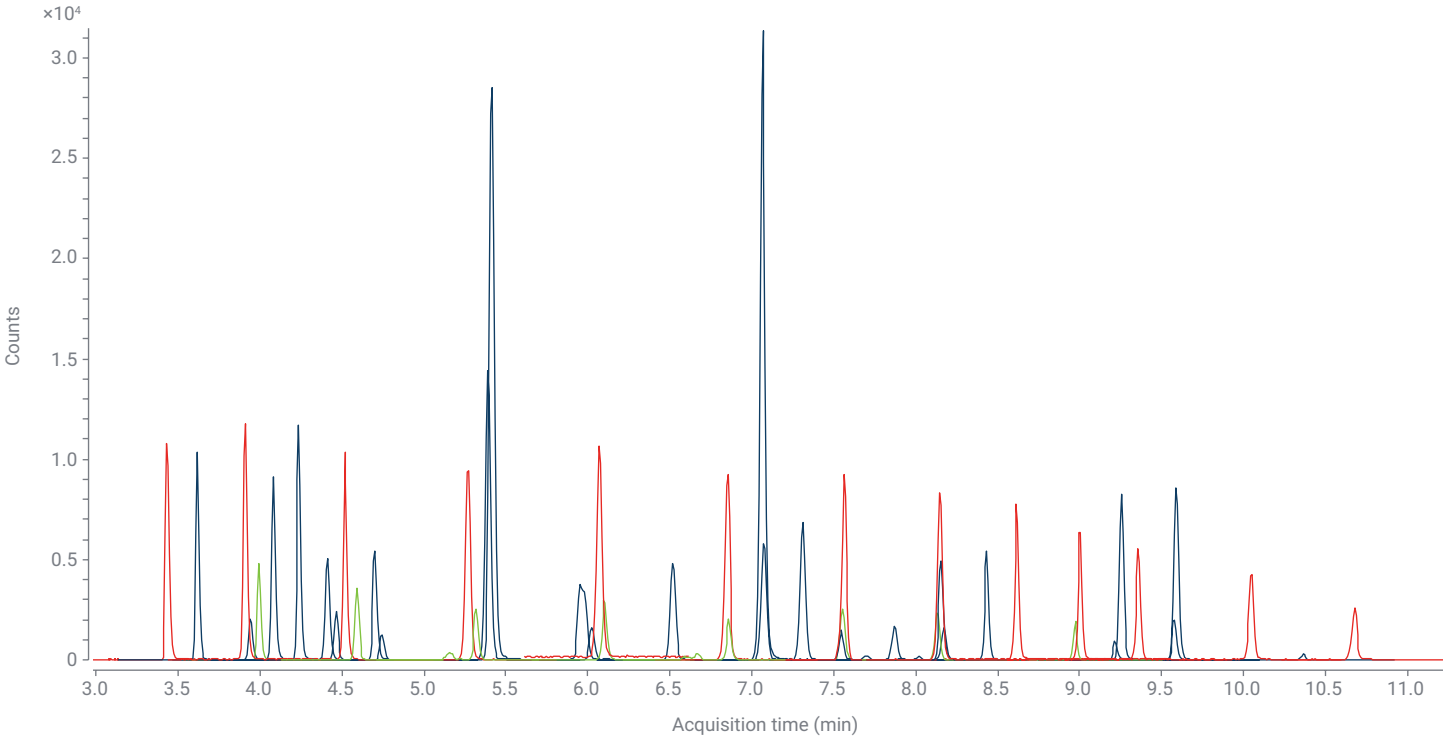

Figure 11. Mixture of 47 PFAS in 90% ACN, 9.6% MeOH, 0.4% water (red: PFCAs, green: PFSAs, blue: others; quantifier transitions; 5 µL injection volume; feed injection).

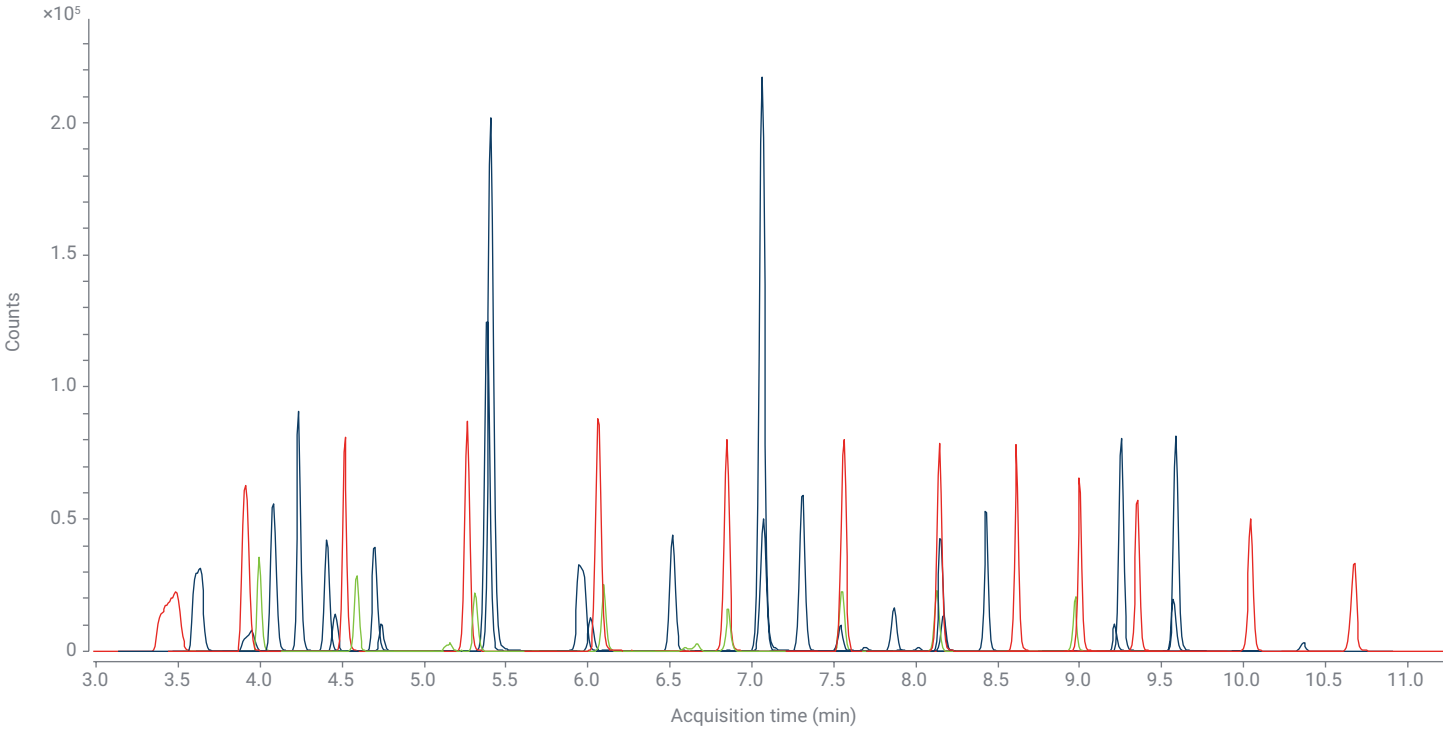

Figure 12. Mixture of 47 PFAS in 90% ACN, 9.6% MeOH, 0.4% water (red: PFCAs, green: PFSAs, blue: others; quantifier transitions; 40 µL injection volume; feed injection).

Conclusion

Feed injection can reduce solvent effects significantly. Its use improves peak shapes and avoids issues with automatic integration. Due to the increased peak intensities, lower detection limits become available. The increased repeatability enables more reliable quantification. Branched isomer detection and quantification are not impacted.

References

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